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THE EXPOSURE OF HUMANS TO *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* THROUGH FOOD AND DRINKING WATER IN FINLAND AND MINNESOTA, USA – A LITERATURE REVIEW

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Abstract

The aim of this licentiate thesis is to assess by literature the potential exposure of humans to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) through food and drinking water in Finland and Minnesota, USA and to represent the prevalence (the total number of cases of a disease at a specific time) of Johne's disease and the prevalence and incidence (the number of new cases of a disease during a certain period of time) Crohn's disease in the United States, Minnesota and Finland.

Johne's disease (JD), also known as paratuberculosis, is a globally important chronic intestinal disease of cattle and other ruminants such as goats and sheep caused by MAP.

Crohn's disease (CD) is a chronic intestinal disease of humans. The etiology of CD is unknown but in addition to genetic susceptibility, environmental factors have been found to have an impact on the onset of the disease. It has been suggested that MAP could be one of the etiologic agents of CD.

In the United States, JD is more common in dairy cattle than in beef cattle. The apparent cow-level prevalence is 6% and apparent herd-level prevalence is 68% in dairy cattle. In Minnesota the apparent prevalence of JD in dairy cattle at the cow-level is 3% and at the herd-level 46%. In beef cattle the prevalence at the cow-level is only 0.3% in Minnesota. The prevalence of CD in the United States is 241/100,000 and the annual incidence 20/100,000. The prevalence of CD in Minnesota is 222/100,000 and the annual incidence 13/100,000.

In Finland, JD has been diagnosed in five beef cattle herds since 1992. The disease has not been diagnosed in dairy cattle or sheep or goats in Finland. The prevalence of CD in Finland is 124/100,000 and the annual incidence 9/100,000.

The prevalence of MAP in food and drinking water in Finland has not been studied. Despite this, it is unlikely that people are exposed to MAP through drinking water or by eating foods of Finnish origin because the prevalence of JD in Finland is very low. However, exposure to the bacterium is possible by eating imported beef and dairy products such as cheese and yogurt. The share of imported foods within these food groups is relatively large in Finland. Dairy products and beef are imported for example from Germany and Denmark where the prevalence of JD at the herd-level is about 50–80%.

In the United States the occurrence of MAP in foods and drinking water has been studied quite much. It appears that the bacterium is found in foods and drinking water of U.S. origin. Because JD is so common in the United States and Minnesota, it is likely that people are exposed to the bacterium in Minnesota even though not all the food eaten is produced in the state.

It is likely that people in areas of high prevalence of JD are exposed more to MAP than people in areas of low prevalence of JD. Comparing subsets of CD patients with high exposure to MAP to healthy controls with and without exposure to MAP could reveal the possible role of MAP in the complex etiology of CD. Based on this literature review it can be assumed that in Finland CD is caused by some other environmental agent than MAP. This licentiate thesis sets up further research needs to estimate the true human exposure to MAP.

Keywords
Mycobacterium avium subsp. *paratuberculosis*, Johne's disease, paratuberculosis, Crohn's disease, food, drinking water

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Ihmisten altistuminen *Mycobacterium avium* subspecies *paratuberculosis* -bakteerille elintarvikkeiden ja juomaveden välityksellä Suomessa ja Minnesotan osavaltiossa, Yhdysvalloissa – kirjallisuuskatsaus

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Tiivistelmä – Referat

Tämän lisensiaatin tutkielman tarkoituksena on arvioida kirjallisuuden avulla ihmisten altistumista Suomessa ja Minnesotan osavaltiossa, Yhdysvalloissa *Mycobacterium avium* subspecies *paratuberculosis* (MAP) -bakteerille elintarvikkeiden ja juomaveden välityksellä sekä raportoida paratuberkuloosin prevalenssi eli vallitsevuus (sairaiden lukumäärä tietyllä hetkellä) ja Crohnin taudin prevalenssi ja insidenssi eli ilmaantuvuus (uusien sairastapauksien määrä tiettynä ajanjaksona) Yhdysvalloissa, Minnesotassa ja Suomessa.

Paratuberkuloosi (englanninkielisessä kirjallisuudessa usein Johnen tauti) on maailmanlaajuisesti merkittävä nautojen ja muiden märehtijöiden kuten vuohien ja lampaiden krooninen suolistosairaus, jonka aiheuttaa MAP -bakteeri.

Crohnin tauti puolestaan on ihmisten krooninen suolistosairaus. Crohnin taudin etiologia eli sairauden syy on epäselvä, mutta geneettisen alttiuden lisäksi myös ympäristötekijöillä on todettu olevan vaikutusta taudin puhkeamiseen. On ehdotettu, että MAP saattaisi olla yksi Crohnin taudin aiheuttajista.

Yhdysvalloissa paratuberkuloosi on yleisempi lypsykarjoissa kuin lihakarjoissa. Lypsylehmillä prevalenssi on yksilötasolla 6 % ja tilatasolla 68 %. Minnesotassa lypsylehmillä paratuberkuloosin prevalenssi on yksilötasolla 3 % ja tilatasolla 46 %, kun taas osavaltion lihanaudoilla prevalenssi on ainoastaan 0,3 %. Crohnin taudin prevalenssi on Yhdysvalloissa 241/100.000 ja vuosittainen insidenssi 20/100.000. Minnesotassa Crohnin taudin prevalenssi on 222/100.000 ja vuosittainen insidenssi 13/100.000.

Suomessa paratuberkuloosia on todettu viidellä eri lihakarjatilalla vuoden 1992 jälkeen. Tautia ei ole löydetty lypsykarjoista eikä myöskään vuohilla tai lampailla. Crohnin taudin prevalenssi on Suomessa 124/100.000 ja vuosittainen insidenssi 9/100.000.

Suomessa MAP-bakteerin esiintyvyyttä elintarvikkeissa ja juomavedessä ei ole tutkittu. Siitä huolimatta on epätodennäköistä, että ihmiset altistuisivat bakteerille juomaveden kautta tai syödessään kotimaisia elintarvikkeita, koska paratuberkuloosin prevalenssi Suomessa on erittäin alhainen. Bakteerille altistuminen on kuitenkin mahdollista esimerkiksi ulkomaalaisten maitotuotteiden, kuten juuston ja jogurtin sekä naudanlihan välityksellä, joiden maahantuonti ja osuus kulutetuista elintarvikkeista Suomessa on kohtalaisen suurta. Maitotuotteita ja naudanlihaa tuodaan Suomeen paljon muun muassa Saksasta ja Tanskasta, missä paratuberkuloosin tilatason prevalenssi on noin 50–80 %.

Yhdysvalloissa MAP-bakteerin esiintyvyyttä elintarvikkeissa ja juomavedessä on tutkittu kohtalaisesti. Vaikuttaa siltä, että bakteeria esiintyy Yhdysvalloissa juomavedessä sekä elintarvikkeissa. Koska paratuberkuloosi on niin yleinen Yhdysvalloissa ja Minnesotassa, on todennäköistä, että ihmiset Minnesotassa altistuvat bakteerille, vaikkei kaikki minnesotalaisten syömä ruoka olisikaan tuotettu tämän osavaltion alueella.

On todennäköistä, että korkeilla paratuberkuloosin prevalenssialueilla asuvat ihmiset altistuvat MAP-bakteerille enemmän ruoan ja juomaveden välityksellä kuin ihmiset alhaisilla prevalenssialueilla. Vertailemalla Crohnin taudin potilaiden osajoukkoja terveisiin kontrolleihin, jotka sekä altistuvat, että eivät altistu MAP-bakteerille on mahdollista selvittää MAP-bakteerin mahdollista roolia Crohnin taudin monitahoisessa etiologiassa. Tämän kirjallisuuskatsauksen perusteella voidaan olettaa, että Suomessa Crohnin taudin aiheuttaa jokin muu ympäristötekijä kuin MAP. Tämä lisensiaatin tutkielma antaa pohjatietoa todellisen MAP-bakteerille altistumisen arvioinnille.

Avainsanat – Nyckelord

Mycobacterium avium subsp. *paratuberculosis*, Johnen tauti, paratuberkuloosi, Crohnin tauti, elintarvikkeet, juomavesi

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ABBREVIATIONS

CD – Crohn's disease

cfu – Colony forming units

ELISA – Enzyme-Linked Immunosorbent Assay

HTST pasteurization – High temperature short time pasteurization

IBD – Inflammatory bowel disease

JD – Johne's disease

MAC – *Mycobacterium avium-intracellulare* complex

MAP – *Mycobacterium avium* subspecies *paratuberculosis*

MBAH – Minnesota Board of Animal Health

NACMCF – National Advisory Committee on Microbiological Criteria for Foods

OR – Odds ratio

PCR – Polymerase chain reaction

UC – Ulcerative colitis

USDA – United States Department of Agriculture

WHO – The World Health Organization

1 INTRODUCTION

Johne's disease (JD), also known as paratuberculosis is a chronic intestinal disease of cattle and other ruminants such as goats and sheep. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the causative agent of JD. Crohn's disease (CD) is a chronic intestinal disease of humans quite similar to JD. The etiology of CD is unknown but genetic and environmental factors are thought to play a role in the development of the disease. It has been suggested that MAP could be one of the etiologic agents of CD. One possible way for humans to be exposed to MAP is through food and drinking water, since MAP is shed in milk and feces of infected animals and can thus enter the food chain.

If MAP is found to be a causative agent of CD, the occurrence of MAP in foods would pose a big public health risk. It would mean that the human exposure to MAP, not only through foods but through other sources as well, should be limited. This would be challenging since JD is so widespread throughout the world. Proving that MAP is one of the etiologic agents of CD would also cause significant changes in the animal husbandry including the control of JD in cattle production, cattle import and export and the way food is produced. It would also significantly increase the amount of research done in these two diseases and may help developing a cure for CD. Researching this possible link between JD and CD would thus be crucial.

2 AIM OF THE STUDY

The aim of this licentiate thesis is to evaluate the possible link between MAP and CD, to report the exposure of humans to MAP through food and drinking water in Finland and Minnesota, USA and also to represent the prevalence of JD and the prevalence and incidence of CD in these geographical areas. This focus is due to that JD is very common in cattle, especially in dairy cattle in Minnesota and really rare in Finland. CD on the other hand is common in both areas.

The hypothesis is that people are exposed to MAP less in Finland than in Minnesota through food and drinking water due to the different prevalence of JD in these areas. The exposure of humans to MAP through food and drinking water has not been compared geographically like this before. This thesis sets up further research needs to estimate the true exposure of humans to MAP in Finland and Minnesota. The possible role of MAP in the etiology of CD could be further revealed if subsets of CD patients with high exposure to MAP were identified and compared to controls with and without exposure to MAP. This kind of research would be a small step towards unraveling the etiology of CD and possibly eventually finding a cure for the disease.

3 JOHNE'S DISEASE

JD affects a large number of ruminant species including cattle, sheep, goats and deer (Cocito *et al*. 1994). It has also been diagnosed in ruminants such as Rocky Mountain bighorn sheep (*Ovis canadensis*), Rocky Mountain goat (*Oreamnos americanus*) and wild bison (*Bison bison athabascae*) (Williams & Spraker 1979, Sibley *et al*. 2007). Positive cases among non-ruminant species such as stumptail macaque monkeys (*Macaca arctoides*), fox (*Vulpes vulpes*), stoat (*Mustela erminea*), weasel (*Mustela nivalis*), crow (*Corvus corone*) and rabbit (*Oryctolagus cuniculus*) have also been found (McClure *et al*. 1987, Beard *et al*. 2001, Judge *et al*. 2006).

3.1 Etiology

JD is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), which belongs to the genus *Mycobacterium* (Cocito *et al*. 1994). There are about 100 members in the genus but most of the members are saprophytic bacteria found in the environment (Hirsh & Biberstein 2004). Mycobacteria are cytochemically gram-positive but they do not stain with the Gram stain because the high lipid and mycolic acid content of their cell walls prevent the uptake of the stains (Quinn *et al.* 2011). The pathogenic members of the genus *Mycobacterium* include bacteria like *M. tuberculosis*, *M. bovis*, *M. avium* and *M. leprae*, which cause chronic granulomatous disease such as tuberculosis in humans, bovine tuberculosis, avian tuberculosis and leprosy, respectively (Quinn *et al*.

2011). Although the pathogenic mycobacteria usually have a specific host, they are capable of infecting other species as well, e.g. *M. bovis* can cause tuberculosis in humans (Quinn *et al*. 2011).

3.1.1 *Mycobacterium avium* subsp. *paratuberculosis* (MAP)

MAP is a rod-shaped, acid-fast, obligate aerobe intracellular bacterium which belongs to the genus *Mycobacterium* (Hirsh & Biberstein 2004, Quinn *et al*. 2011). MAP can be classified as a member of *Mycobacterium avium* complex (MAC) which consists of closely related species *M. intracellulare* and *M. avium* (Goodfellow & Magee 1998). *M. avium* can be divided into four subspecies *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *silvaticum* and *M. avium* subsp. *hominissuis* (Springer *et al*. 1996, Mijs *et al*. 2002). There are at least two different main strain types of MAP; the cattle (C) and sheep (S) strains (Collins *et al*. 1990). Since these strains are not host-specific, another classification has been developed for MAP (type I and type II) to avoid confusion with the names, since it is not always evident whether the name "sheep" or "cattle" stain refers to the host where the bacterium was isolated or to the strain of bacterium in question (Stevenson *et al*. 2002). Type I is comprised of four pigmented ovine isolates of MAP and type II comprises nine isolates from a broad host range including humans (Stevenson *et al*. 2002, Dohmann *et al*. 2003). The pigmented isolates produce yellow or orange pigment which is present in all stages of growth in the bacterium (Stevenson *et al*. 2002).

3.2 Global prevalence

JD is common throughout the world (Harris & Barletta 2001). However, there are areas where the disease is really rare, such as in the eastern regions of Australia and in Finland, Sweden, Norway and Iceland (Nyberg *et al*. 2005, Evira 2008, Nacy & Buckley 2008). For example, in Sweden only about 50 positive cases of JD in beef cattle have been found since 1993 (Lewerin 2007). In Norway, the disease is endemic in goats in some parts of western Norway (Norwegian Veterinary Institute 2014). In cattle, only few positive cases have been found since mid-19th century in Norway (Nyberg *et al*. 2005). In Denmark, in contrast to the other Nordic countries, JD is common in dairy cattle (Nielsen 2007). At the herd-level, the prevalence is 47% in Denmark (Nielsen

2007). In Germany the herd-level prevalence of JD in cattle is over 80% and on cow-level between 1% and 17% in several states (Bülte *et al*. 2005). JD was thought to be eradicated from Poland in the 1960–70s but in a study made in 2008, the apparent herd-level seroprevalence of JD in dairy cattle herds was found to be 6% in two districts of northeastern Poland (Szteyn & Wiszniewska-Łaszczych 2011). The cow-level seroprevalence was 2% (Szteyn & Wiszniewska-Łaszczych 2011). In Brazil, the disease occurs sporadically and clinical cases of bovine JD have been found in several states in the 21st century (Gomes 2010).

3.3 Clinical signs

The clinical signs in cattle include diarrhea, weight loss, and in advanced disease cachexia and reduction in milk yield (Whitlock & Buergelt 1996). Also "bottle jaw" (intermandibular edema) can occur in advanced stages of the disease because of hypoproteinemia due to lesions in the intestines (Whitlock & Buergelt 1996). JD has an incubation period of two to ten years (Whitlock & Buergelt 1996). In an infected herd, most animals do not show any clinical signs and in general, young animals are normally asymptomatic (Whitlock & Buergelt 1996). In adult animals the infection might be subclinical and it is possible that these animals shed the bacteria in feces (Whitlock & Buergelt 1996). An animal with clinical signs can shed billions of bacteria in feces daily (Sweeney 1996).

In sheep and goats the clinical signs appear earlier than in cattle (Stehman 1996). A common clinical sign in sheep and goats is emaciation (Radostits *et al*. 2007). Shedding of the wool in sheep might be possible (Radostits *et al*. 2007). Diarrhea is not a common sign in sheep and goats (Radostits *et al*. 2007). Depression and dyspnea can occur in goats but these signs are not normally evident in sheep (Radostits *et al*. 2007).

3.4 Pathogenesis and pathologic findings

The ingested bacteria enter the Peyer's patches in the ileum through cells called M-cells (Momotani *et al*. 1988). Within the Peyer's patches the bacteria are phagocytized by macrophages (Momotani *et al*. 1988). The bacteria multiply within the macrophages and granulomas form to the site of entry (Zurbrick & Czuprynski 1987, Cocito *et al*. 1994).

Gross lesions of JD in cattle are normally confined to the distal part of the small intestine and associated lymph nodes (Whitlock & Buergelt 1996). The pathologic findings in cattle include cachexia, chronic enteritis, chronic intestinal lymphangitis and mesenteric and ileocaecal lymphadenopathy (Buergelt *et al*. 1978). The mucosa of ileum seems visibly thickened and corrugated (Buergelt *et al*. 1978). In histopathology, epitheloid macrophages and multinucleate giant cells in the lamina propria and submucosa of the infected gut are found (Buergelt *et al*. 1978). Also microgranulomas in the liver can be found (Buergelt *et al*. 1978). The villi of the mucosa of the small intestine often fuse which causes malabsorption of nutrients and can eventually lead to emaciation of the animal (Whitlock & Buergelt 1996, Radostitis *et al*. 2007).

3.5 Transmission

Calves become infected with MAP by vertical transmission *in utero* or by orally ingesting the bacterium by drinking contaminated milk, colostrum or water or by ingesting feces contaminated with the bacterium for example by sucking contaminated teats (Lawrence 1956, Merkal *et al*. 1987, Seitz *et al*. 1989, Sweeney 1996, Whittington *et al*. 2005). Often the calving pen is contaminated with the bacterium either by the dam or by previous occupants and thus provides an easy route of infection (Sweeney 1996). The bacterium has also been cultured from the semen and genital organs of infected bulls with clinical signs of JD (Larsen *et al*. 1970).

Young animals are more susceptible to JD than adult animals (Sweeney 1996). Because of the long incubation period of the disease, animals infected as adults rarely develop a clinical disease because they are normally culled before the appearance of clinical signs (Whitlock & Buergelt 1996). Adults may become infected by the fecal-oral route for example by eating contaminated feed (Lombard 2011). Also new animals from an

infected herd might infect animals without JD (Sweeney 1996). Minor risks of infection include purchase of contaminated milk or colostrum and sharing of pastures and water sources with animals from infected herds (Lombard 2011).

3.6 Diagnosis, treatment and prevention

3.6.1 Diagnosis

Enzyme-linked immunosorbent assay (ELISA) is used to diagnose JD (Yokomizo *et al*. 1983). Commercially available ELISA are for example milk and serum ELISA tests (Nielsen 2010). The sensitivity of ELISA varies quite much but in general it is low (Nielsen & Toft 2008). Sweeney *et al*. (1995) showed that the sensitivity is only 15%, if ELISA is used to detect subclinical animals. The advantage of ELISA is the low cost of the test (Nielsen 2010). Also the test can easily be performed on a large number of samples and the results are ready quickly (Nielsen 2010).

It was previously thought that an insertion element known as IS*900* was specific to MAP, and that it could be used for the diagnosis of JD with polymerase chain reaction (PCR) (Collins *et al*. 1989, Green *et al*. 1989, Vary 1990, Moss *et al*. 1991). It was later noticed that the insertion element is not specific to MAP but it was also occasionally found in other Mycobacteria species (Cousins *et al*. 1999, Englund *et al*. 2002). Despite the possible cross-reaction with IS*900*-like elements, IS*900* is recommended as a target gene for MAP especially if complimentary testing is done (Bölske & Herthnek 2010). This is based on the thorough validation of the gene as a target gene (Bölske & Herthnek 2010). Other ways to diagnose MAP by PCR are for example the detection of DNA fragments f57 and *hspX* (Poupart *et al*. 1993, Ellingson *et al*. 1998).

Besides the extensive use of PCR for detection of MAP, the bacterial culture is still used as a confirmatory test to diagnose the disease (Chiodini *et al*. 1984, Möbius *et al*. 2008). By bacterial culture, MAP can be distinguished from other members of MAC bacteria by its requirement of mycobactin for growth (Thorel *et al*. 1990). The bacterium can be cultured either from fecal or tissue samples (Collins 1996). Problems with bacterial culture are for example the cost of the test and the requirement to decontaminate the sample to kill all the other bacteria, if fecal samples are used (Collins 1996). Also MAP requires at least 12–16 weeks of incubation before a reliable positive

result can be read (Collins 1996). A cost-effective way to detect a MAP infected herd is to use pooled fecal samples tested by bacterial culture (Wells *et al*. 2003). Pooled fecal samples are most sensitive in herds with animals which shed moderate to high amounts of the bacterium (Wells *et al*. 2003).

3.6.2 Treatment and prevention

There is no definitive cure for infections with MAP or for JD (Fecteau & Whitlock 2011). In the United States, there are no drugs allowed to be used for food animals in the treatment of JD (Fecteau & Whitlock 2011). Culling of clinical cases of JD is recommended (Garry 2011). Vaccination might be used to help control JD in heavily infected herds where other control methods have been unsuccessful (Whitlock 2010). Currently vaccination is not used in any country to control JD in general (de Lisle 2010). Problems with the vaccine are the possible false-positive results in bovine tuberculin test (de Lisle 2010).

The best treatment option for JD is to prevent the disease of spreading to the farm (Fecteau & Whitlock 2011). For a herd free of JD, the most important way to remain that status is to buy new animals from JD free herds and animals that have been tested for the disease (Wells *et al*. 2000). A primary method JD can be controlled at the farm level is through implementation of a JD control program utilizing assessment and herd management plans (USDA 2010). Also important in controlling JD at the farm level is to prevent exposure of susceptible animals such as calves to the bacterium and to identify and cull infected animals from the herd (Garry 2011).

4 JOHNE'S DISEASE IN FINLAND

In Finland, JD is a notifiable disease and veterinarians have to report positive cases to the Regional State Administrative Agencies (Decree of the Ministry of Agriculture and Forestry 1010/2013, Section 1). When positive cases are found, no restrictions are given to the farms (Seuna & Seppänen 2003). Also all control measures at the farm level are voluntary (Seuna & Seppänen 2003).

4.1 Testing and positive cases

Before the 1990's, the last reported case of JD in Finland was in 1918 (FAO 1990). In the early 1990's, it was assumed that Finland was free of JD and testing of animals was started to attain a free country status (Seuna & Seppänen 2003) The first positive clinical case in over 70 years was found at the end of 1992 (Hintikka & Seuna 1998, Seuna & Seppänen 2003). In 1993–1994, practically all beef breeder herds in Finland were tested for JD by ELISA with a total of 2,893 samples (Hintikka & Seuna 1998, Seuna & Seppänen 2003). Of those, 35 serum samples (1%) were positive by ELISA but the results were not confirmed by bacterial culture (Hintikka & Seuna 1998, Seuna & Seppänen 2003). In 1994, 678 blood samples mostly from dairy cattle were tested and eight of those samples (1%) were positive by ELISA (Hintikka & Seuna 1998). Three of these positive samples were also positive with complement fixation method (Hintikka & Seuna 1998). In 1995–1996 the quantities of samples tested were low. Four positive samples were found in 1995 by ELISA and two in 1996 (Hintikka & Seuna 1998). In 1992–1996, 189 samples from sheep were tested by CF method and no positive cases were found (Hintikka & Seuna 1998).

Since October 2005, Finnish Food Safety Authority has had a project to find out the prevalence of clinical cases of JD in Finland. Producers and veterinarians can send samples of animals with clinical signs to be analyzed for JD free of charge. The samples can be fecal or blood samples from live cattle or organ sample from slaughterhouses or from necropsies. Approximately 10 samples per year have been sent thus far. No positive results have been found at the time of publication of this thesis. The project is still in progress. (Seppänen J, Finnish Food Safety Authority Evira, personal communication, March 2014).

In total, five beef herds were found positive for JD in Finland between 1992 and 2000 (Evira 2007). Also two positive cases in farmed wild ruminants were found in 2003 and 2007 (Evira 2007).

4.2 Risk of Johne's disease in Finland

A risk assessment of JD in Finnish suckler herds has been made in 2004 (EELA 2004). The risk assessment report notes that the biggest risk of JD to spread into a herd in Finland is by bought animals (EELA 2004). Imported animals pose a bigger risk than those of domestic origin (Seuna & Seppänen 2003). The Association for Animal Disease Prevention Organization (ETT) provides instructions for producers importing cattle (ETT 2014b). The imported animals must be examined for JD by ELISA or bacterial culture. The tests must be completed before the cattle are imported into Finland (Seuna & Seppänen 2003).

Control measures for JD in Finland at a national level could include establishing a voluntary control program, including the disease in the list of notifiable diseases, educating producers and veterinarians about the disease, providing more funds into controlling the disease (EELA 2004). The producers should also follow the import instructions of ETT (EELA 2004).

5 JOHNE'S DISEASE AND CONTROL PROGRAMS IN THE UNITED STATES

5.1 Dairy cattle

The National Animal Health Monitoring System (NAHMS) is a program within the United States Department of Agriculture (USDA) that conducts nationwide studies to provide information about the health and management of U.S. livestock and poultry (USDA 2014). The Dairy 1996 study, which represented 79% of the U.S. dairy cow population in 20 states, found the adjusted cow-level seroprevalence of JD to be 3% tested with ELISA (NAHMS 1997). At the herd-level, the apparent prevalence was 22% (NAHMS 1997).

In the Dairy 2002 study, a total of 7,272 fecal samples were taken from 62 operations in 20 states of which 9% were positive by bacterial culture (NAHMS 2002). In the study, 15,167 milk samples were also taken in 17 states and the samples were tested by milk ELISA and 3% of the samples were positive (NAHMS 2002). In total, 19,378 serum samples from 106 operations were tested in 21 states with a commercially available serum ELISA and of those samples, 6% were positive or strong positive (NAHMS 2002). These positive serum ELISA results were confirmed by milk ELISA and 46% of the cases were positive (NAHMS 2002). The study also included environmental samples from 98 farms which were taken from sites where the manure accumulates from a majority of adult cattle (NAHMS 2002). At least one positive sample was found on 70% of the study farms (NAHMS 2002).

In the Dairy 2007 study, environmental samples were collected from 524 operations in 17 states representing 80% of U.S. dairy operations and 83% of U.S. dairy cows (APHIS 2007). From each operation, six combined manure samples were taken. MAP was isolated from at least one sample on 68% of the farms (APHIS 2007).

5.2 Beef cattle

The Beef ‘97 study estimated the seroprevalence of JD in U.S. beef cattle (Dargatz *et al*. 2001). In total 10,371 blood samples were taken on 380 operations in 21 states and of these, 40 samples (0.4%) were found positive and 30 herds (8%) had at least one seropositive animal tested by ELISA (Dargatz *et al*. 2001).

In a study made in Texas in 2000–2001, samples from 4,579 beef cattle from 115 ranches were taken (Roussel *et al*. 2005). The blood samples were tested by ELISA and 137 (3%) of them were positive and 50 (44%) of the herds had at least one seropositive animal (Roussel *et al*. 2005).

5.3 Sheep

The Sheep 2001 study covered sheep producers from 22 states represented 42% of the sheep producers and 93% of ewes in the country (NAHMS 2003). Data were collected from 1,101 operations that had 20 or more ewes and the producers were asked which diseases were present (suspected or confirmed) on the farms during the previous 3 years (NAHMS 2003). JD was reportedly present on 2% of the farms but only 33% of these cases were confirmed by laboratory testing (NAHMS 2003).

5.4 Minnesota

Wells *et al*. (2008) gathered information from Minnesota Board of Animal Health (MBAH) database of herds participating in the Voluntary Bovine Minnesota Johne's Disease Control Program (MNJDCP) which is a state-level JD control program. They found that in Minnesota in 2001 in dairy herds, the apparent cow-level seroprevalence of JD was 9% and in beef herds 5% (Wells *et al*. 2008). By 2006 the seroprevalence had decreased to 3% in dairy herds and 0.3% in beef herds (Wells *et al*. 2008). The participation of producers in the program increased during the study (Wells *et al*. 2008). In 2001, 146 dairy herds and 11 beef herds were included in the prevalence estimation, and in 2006, 380 dairy herds and 52 beef herds of herds participated in the study (Wells *et al*. 2008).

In a study made in Goodhue County, Minnesota, 157 dairy herds, of which JD infection status was known for 125 herds, were tested for JD (Wells SJ, unpublished). Both fecal and blood samples were taken from the environment and from individual animals (Wells SJ, unpublished). Of the herds, 27 were positive by PCR and 30 herds by bacterial culture so the detected herd-level prevalence was thus 46% (Wells SJ, unpublished).

5.5 Johne's disease control programs in the United States

In the United States, all the control programs are voluntary to producer participation (Ferrouillet *et al*. 2009, USDA 2010). Each state has its own program and the programs have different levels of participation with different requirements (USDA 2010). The herds can be classified into six different levels within the program depending on their test results, number of positive animals and years in the program (USDA 2010).

In addition to the Minnesota JD Control Program, in Minnesota there is a voluntary JD Demonstration Herd Project whose purpose is to demonstrate whether the use of herd management practices would be able to control and reduce the transmission of the disease (Ferrouillet *et al*. 2009).

6 CROHN'S DISEASE

Inflammatory bowel disease (IBD) encompasses two forms of chronic intestinal inflammation, ulcerative colitis (UC) and Crohn's disease (CD) (Podolsky 1991). CD can occur in people at any age but typically the disease is diagnosed in teenagers and young adults (Hanauer *et al*. 2001). UC and CD share many similarities in epidemiology and clinical signs but they are thought as two distinct diseases (Sands & Siegel 2010). UC is confined to the large bowel and CD can be found in the entire GI tract (Sands & Siegel 2010, Osterman & Lichtenstein 2010). Also the inflammation in UC is diffuse and in CD it is more focal (Osterman & Lichtenstein 2010, Sands & Siegel 2010). Skip lesions (patchy areas of inflammation) and granulomas are also distinctive in CD (Sands & Siegel 2010).

6.1 Etiology

The etiology of JD is not known but it is believed to be multifactorial (Sartor 2006). Genetic susceptibility, environmental triggers, luminal microbial antigens and adjuvants and immune response of the individual all have a role in the development of the disease (Sartor 2006). A person with a first- or a second-degree relative with inflammatory bowel disease has a 10–15% chance of getting the disease themselves (Stenson & Korzenik 2003). Studies done with monozygotic twins have found concordance rates for CD to be around 50% (Orholm *et al*. 2000, Halfvarson *et al*. 2003). Genes specifically associated with CD are for example *NOD2/CARD15*, *ATG16L1* and *IRGM* (Russel *et al*. 2004, Cho 2008). Several risk factors associated with the development of CD have also been suggested, including smoking, infectious agents such as MAP and adherent-invasive *Escherichia coli*, socio-economic status and diet (Chiodini *et al.* 1984, Somerville *et al*. 1984, Barnich & Darfeuille-Michaud 2007, Carbonnel *et al*. 2009).

Also other, unknown environmental factors may have an impact in the development of the disease. Clusterings of CD have been found for example in Mankato, Minnesota where seven out of 285 students of the Mankato High School Class of 1980 had CD after 19 years of graduation (Van Kruiningen & Freda 2001). None of the CD patients were related to each other and all of the patients had been swimming in a pond and lake in Mankato (Van Kruiningen & Freda 2001). Also extraordinarily high coliform counts were measured from Blue Earth River in Mankato (Van Kruiningen & Freda 2001). Blue Earth River provides 75% of the drinking water in Mankato (Van Kruiningen & Freda 2001). Another cluster of CD has been found in Gloucestershire, England in 1986 (Allan *et al*. 1986). In a town with 1,800 people, twelve patients of CD were found and only two of them (a father and daughter) were related (Allan *et al*. 1986). Pickup *et al*. (2005) suggested an environmental exposure to MAP in South Wales, United Kingdom where in an area with endemic JD an increase in CD had been noticed in the population of 11 districts directly bordered or immediately adjacent to the river Taff. The catchment area of the river contains 1,013 farms with a total of 30,435 cattle. MAP DNA was detected by PCR in 33% (31/96) of Taff river water samples taken within one year (Pickup *et al*. 2005). The authors also propose exposure to MAP through wind and aerosols based on the geographical location of the districts with the increase of JD compared to the river Taff (Pickup *et al*. 2005).

6.2 Global prevalence and incidence

The prevalence and incidence of CD is high in Europe and North America (Stone *et al*. 2003, Loftus 2004, Bernstein *et al*. 2006, Manninen *et al*. 2010). In Stockholm County, Sweden the prevalence of CD was 213 per 100,000 in 2002 and the incidence was 8 per 100,000 in 1990–2001 (Lapidus 2006). In Denmark the prevalence of CD was 151 per 100,000 and the incidence in women 11 per 100,000 and in men 9 per 100,000 in 1998–2002 (Jacobsen *et al.* 2006). In Germany, large nationwide studies have not been conducted but in a study in rural southern Germany the incidence of CD was 7 per 100,000 in 2004−2006 (Ott *et al*. 2008). Among the highest prevalence and incidence of CD ever reported are the results from the Canadian province of Nova Scotia with prevalence of 319 per 100,000 in 1998–2000 and annual incidence of 20 per 100,000 in 1998–2000 (Bernstein *et al*. 2006). In contrast, the incidence and prevalence of CD are lower in South America and Asia although the figures are increasing (Loftus 2004). For example the incidence was 0.9 per 100,000 in Korea in 2001–2005 and 0.7 in Uruguay in 2007– 2008 (Yang *et al*. 2008, Buenavida *et al.* 2011).

6.3 Clinical signs

CD is characterized by acute and chronic inflammation of the small and large intestine and other parts of the gastrointestinal tract (Lashner 2000). CD can be found in the entire gastrointestinal tract (Stenson & Korzenik 2003). Depending on the site of the disease the symptoms include abdominal pain, weight loss, steatorrhea, diarrhea, hematochezia and fever (Lashner 2000).

6.4 Pathogenesis and pathologic findings

The most widely agreed theory of the pathogenesis of inflammatory bowel disease is that commensal enteric bacteria excessively activate T cells to develop aggressive immune responses which will ultimately lead to chronic intestinal inflammation in genetically susceptible humans (Sartor 2006). Environmental factors are required in and enhance this activation (Sartor 2006). Also commensal bacteria of the gut are believed to play a role in the pathogenesis of IBD since it has been noted that in transgenic mice, resident enteric bacteria are required for development of spontaneous colitis and that the

diversity of enteric microbiota is reduced by 50% in CD (Sellon *et al.* 1998, Ott *et al*.2004, Sartor 2006).

The most distinctive pathologic finding in CD is focal intestinal inflammation (Sands & Siegel 2010). The inflammation in the intestine is often transmural and the disease can be associated with intestinal granulomas, strictures and fistulas (Abraham & Cho 2009). The iliocecal region of the bowel is affected as an initial site in about 40% of the patients, small intestine in 30% and colon in 25% of patients (Lashner 2000). In one third to one half of all patients the disease affects both ileum and colon (Sands & Siegel 2010).

6.5 Diagnosis and treatment

The diagnosis of CD is based on clinical signs, medical history and on the results of laboratory tests, endoscopy, imaging studies such as CT and MRI and on pathologic findings (Baumgart & Sandborn 2012). With endoscopic studies the visualization of the mucosa of the intestine is possible thus enabling to assess the extent of inflammation in the gut (Sands & Siegel 2010). Ileocolonoscopy with biopsies is used as a golden standard to diagnose CD (Baumgart & Sandborn 2012).

Since CD is a non-curable disease, the aim is to induce and maintain remission and to improve the quality of life of the patient (Sands & Siegel 2010). The treatment is based on medical, nutritional and surgical therapy (Baumgart & Sandborn 2012). Medication used for CD patients varies quite a lot based on the site and activity of the disease (Dignass *et al*. 2010). Common drugs are aminosalicylates such as sulfasalazine; corticosteroids, antibiotics such as metronidazole and immunosuppressive drugs such as azathioprine and mercaptopurine (Sands & Siegel 2010). No specific diet can be recommended for CD patients but since common symptoms of the disease are weight loss and malnutrition, sufficient supplementation of nutrients and calories in the diet is necessary (Sands & Siegel 2010). Smoking promotes fistulising and it might worsen the effect of medical therapy (Baumgart & Sandborn 2012). Surgical therapies include resections, bypass surgery and repair or resection of fistulas but surgery should not be regarded as a primary therapy (Sands & Siegel 2010, Baumgart & Sandborn 2012).

7 CROHN'S DISEASE IN FINLAND

In Finland, all permanent residents are covered by the National Health Insurance scheme which reimburses a share of medical costs and cost of drugs prescribed by a physician (Kela 2014b). Therefore nationwide prevalence and incidence studies are easy to conduct as statistics on the prescription of drugs specific to a certain disease is available (Kela 2014a).

The incidence and prevalence of CD in adults in Finland are represented in Table 1. The incidence of CD in children in Finland is represented in Table 2. Both the incidence and prevalence of CD are increasing in adults and the incidence of pediatric CD is increasing in Finland (Tables 1 and 2).

Table 1 Incidence and prevalence of Crohn's disease in adults in Finland

Study period	Area	Incidence/ 100,000	Prevalence/ 100,000	Reference
1975	Helsinki	1.0	NR[a]	Halme *et al.* 1989
1985	Helsinki	3.0	NR	Halme *et al.* 1989
1986	Tampere	5.0	40.0	Manninen *et al*. 2010
1993	nationwide	NR	38.0	Jussila *et al.* 2013
1999	Tampere	9.4	124.0	Manninen *et al*. 2010
2000–2007	nationwide	9.2	NR	Jussila *et al*. 2012

[a]NR Not reported

Table 2 Incidence of Crohn's disease in children in Finland

Study period	Age range, years	Area	Incidence/ 100,000	Reference
1987	0–17	Helsinki and Tampere	1.7	Turunen *et al*. 2006
1992	0–17	nationwide	2.2	Lehtinen P, personal communication February 2012
1992–2003	0–17	nationwide	3.7	Lehtinen *et al*. 2011
2003	0–17	Helsinki and Tampere	2.6	Turunen *et al*. 2006
2003	0–17	nationwide	5.3	Lehtinen P, personal communication Febryary 2012

8 CROHN'S DISEASE IN THE UNITED STATES

8.1 Adults

Inflammatory bowel disease is not a reportable condition in the United States and there are no national registries for the disease in the country (Kappelman *et al*. 2007). Therefore large population based studies are difficult to execute (Kappelman *et al*. 2007). The Mayo Clinic, established in Rochester, Minnesota is a highly distinguished referral hospital which is ranked highest in gastroenterology in the United States in 2013−2014 (U.S. News & World Report 2014). They have conducted studies on CD since 1940's in Olmsted County, Minnesota (Loftus *et al*. 2007). The incidence and prevalence of CD in adults the United States including Minnesota are represented in Table 3. The incidence of CD in Minnesota has risen dramatically from the 1940's (Table 3). The prevalence of CD in Minnesota has risen from 91/100,000 to 222/100,000 in 25 years (Table 3). Minnesota used to have the highest prevalence numbers of CD in the United States but new research findings show that the incidence and prevalence of CD are increasing in the whole country (Table 3).

Table 3 Incidence and prevalence of Crohn's disease in adults in the United States and Minnesota

Study period	Area	Incidence/ 100,000	Prevalence/ 100,000	Reference
1996–2002	Northern California	6.3	NR[a]	Herrinton *et al*. 2008
1999–2001	nationwide	NR	129.0	Herrinton *et al*. 2007
2002	Northern California	NR	96.0	Herrinton *et al*. 2008
2004	33 states in the U.S.	NR	201.0	Kappelman *et al*. 2007
2009	nationwide	20.0	241.0	Kappelman *et al*. 2011
2009	nationwide, military health care system	NR	180.0	Betterridge *et al.* 2013
1940–1949	Olmsted County, MN	2.3	NR	Loftus *et al*. 2007
1980–1989	Olmsted County, MN	6.8	NR	Loftus *et al*. 2007
2001–2004	Olmsted County, MN	12.9	NR	Ingle *et al*. 2007
1980	Olmsted County, MN	NR	91.0	Gollop *et al*. 1988
2001	Olmsted County, MN	NR	174.0	Loftus *et al*. 2007
2005	Olmsted County, MN	NR	222.0	Ingle *et al*. 2007

[a]NR Not reported

8.2 Children

The incidence and prevalence of CD in children in the United States and Minnesota are represented in Table 4. The prevalence of CD in children is significantly higher in Minnesota than in the rest of the country (Table 4). The incidence in children between Minnesota and the rest of the country is difficult to compare due to the lack of incidence studies in Minnesota (Table 4).

Table 4 Incidence and prevalence of Crohn's disease in children in the United States and Minnesota

Study period	Age range, years	Area	Incidence/ 100,000	Prevalence/ 100,000	Reference
1996–2006	0–17	Northern California	2.7	NR[a]	Abramson *et al*. 2010
2000–2001	0–17	Wisconsin	4.6	NR	Kugathasan *et al*. 2003
2004	2–19	33 states	NR	43.0	Kappelman *et al*. 2007
2006	0–17	Northern California	NR	12.0	Abramson *et al*. 2010
1940–2000	0–19	Olmsted County, MN	2.4	NR	Loftus *et al*. 2007
2001	0–19	Olmsted County, MN	NR	115.0	Loftus *et al*. 2007
2009	0–19	nationwide	12.0	58.0	Kappelman *et al*. 2011
2009	0–19	nationwide	NR	40.0	Betterridge *et al*. 2013

[a]NR Not reported

9 *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* AND CROHN'S DISEASE

The idea of the possible link between MAP and CD was first introduced in 1913, when similar pathological findings in patients of CD and in cattle with JD were discovered (Dalziel 1913). The connection was first experimentally associated in 1984 after the culture of MAP from intestinal tissues of 3 out of 11 CD patients (Chiodini 1984). Since then a lot of speculation has surrounded the question but no definite proof of the causative link has been found (Shanahan & O'Mahony 2005).

9.1 *Mycobacterium avium* subsp. *paratuberculosis* in Crohn's disease patients' tissues

MAP has been found in CD patients' tissue samples in several studies. A systemic review and meta-analysis made by Feller *et al*. (2007) concluded that MAP DNA was found by PCR in CD patients' tissues or blood more often than in controls in 16 of 18 studies. The odds ratio (OR) ranged from 2 to 32 and the pooled OR was 7 in those 16 studies (Feller *et al*. 2007). By ELISA, in 10 of 13 studies, MAP antibodies were more likely to be detected in CD patients than in controls. In these studies, the OR ranged from 1 to 12 with a pooled OR of 2 (Feller *et al*. 2007). In addition to the occurrence of MAP DNA in CD patients' tissues, MAP has also been cultured from blood and breast milk of humans (Naser *et al.* 2000, Naser *et al.* 2004, Mendoza *et al*. 2010).

9.2 Occupational exposure to *Mycobacterium avium* subsp. *paratuberculosis*

Since MAP is shed in the feces of infected animals it is logic to consider the possible exposure to be bigger among people who work with animals, e.g. farm workers and veterinarians than among those with no or limited animal contact. Also the consumption of raw milk among dairy farmers could be presumed to be higher than among normal consumers. Therefore the prevalence of JD and the occurrence of MAP in tissues would be higher among these occupational groups. But this has been proven wrong at least in one study by Qual *et al.* (2010). They studied the occurrence of MAP in tissues of almost 1,500 dairy and beef cattle producers and veterinarians in the United States (Qual *et al.* 2010). Of the farmers, 66% were in contact with cattle infected with MAP and of the veterinarians the figure was 56% (Qual *et al.* 2010). Even though the

prevalence of CD was high among the study group (474/100,000), they didn't find a statistically significant association between the exposure to MAP and CD (Qual *et al.* 2010). A questionnaire study in England didn't find an association between the exposure to JD positive cattle and CD (Jones *et al*. 2006). In India on the other hand, the occurrence of viable MAP in stool samples of animal attendants working with goats suffering from gastrointestinal symptoms was found to be 50% (Singh *et al.* 2011).

9.3 Controversy

The proposed association between MAP and CD has proven to be quite complex and controversial (Sartor 2005). Sartor (2006) suggests that in certain genetically susceptible individuals a persistent pathogen such as MAP could cause CD. There is controversy on whether MAP is only a commensal bacterium accidentally found in human gut tissue or if it is truly a pathogen causing disease (Sartor 2005). Even though MAP has been found in CD patients' tissues in several studies, it is not the case every time. For example Parrish *et al.* (2009) were not able to culture MAP from CD patients' or healthy controls' blood. In a large number of studies, MAP is also found in healthy controls' tissues, but the selection of the control group has not always been optimal (Feller *et al*. 2007). A two-year combination antibiotic therapy study with clarithromycin, rifabutin and clofazimine, antibiotics able to kill mycobacteria, did not find evidence of benefit in the treatment of active JD (Selby *et al.* 2007). The study didn't examine the occurrence of MAP in the study population's tissues. Therefore the efficacy of the treatment is impossible to evaluate correctly because it could be that only a small portion or none at all of the study population were infected by MAP.

10 HUMAN EXPOSURE TO *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*

10.1 Food

National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has assessed the importance of food as a source of exposure to MAP (NACMCF 2010). NACMCF provides scientific advice to U.S. federal food safety agencies (FSIS 2014). MAP is shed in feces and milk of infected animals (Sweeney 1996). Therefore dairy products and meat products originating from infected animals including dairy and beef cattle, sheep and goats may serve as a potential source of exposure to MAP (NACMCF 2010). In their report, NACMCF concluded that milk, especially unpasteurized raw milk, may be a significant source of human exposure to MAP (NACMCF 2010). They also suggest that ground beef may a potential source of MAP (NACMCF 2010). One possible way food may become contaminated with MAP is during processing such as contamination of carcasses in slaughter plants and meat processing plants (Eltholth *et al*. 2009). In addition to meat, also dairy products can become contaminated with MAP during processing e.g. by fecal contamination or by mixing of pasteurized and raw milk (Eltholth *et al*. 2009). Since MAP is shed to feces and is so persistent in the environment, it is possible that runoffs from farms will lead to contamination of irrigation water. This again can lead to contamination of vegetables and fruits (NACMCF 2010).

10.2 Drinking water

Drinking water can become contaminated with pathogenic bacteria if cattle manure is applied to fields as fertilizer (Thurston-Enriquez *et al*. 2005). The bacteria in the manure can enter water resources such as ground, irrigation, surface and recreational waters by rainfall runoffs (Thurston-Enriquez *et al*. 2005).

Ground water accumulates underground in aquifers and it is contained beneath the surface in rocks and soil (Schmoll *et al*. 2006). Aquifers are layers of rock or sediments which are permeable and porous enough to filtrate and store water underground (Schmoll *et al*. 2006). Ground water rarely requires extreme water treatments to be suitable for drinking and sometimes it does not need any treatment at all (Schmoll *et al*.

2006). Ground water is regarded as a relatively microbially safe source of drinking water (Schmoll *et al*. 2006). Surface waters are susceptible to direct contamination but ground water is sheltered by the overlying soil (Schmoll *et al*. 2006). Despite this, fecal contamination of ground water is possible especially if inadequate protection of ground water is not performed (Schmoll *et al*. 2006). Also the size of the pathogen and the fines of the soil influence how well the pathogen can filter into ground water (Schmoll *et al*. 2006).

In the United States in organic production, uncomposted animal manures can be applied to fields 120 days before harvest for crops whose edible parts are in contact with the soil (Code of Federal Regulations, Title 7 Part 205). If the edible parts are not in contact with the soil, manure can be applied on fields 90 days before harvest. Otherwise the manure must be composted (Code of Federal Regulations, Title 7 Part 205). There are no federal or state rules on application of manure in non-organic production (FDA 2014). In the European Union, the Council Regulation on organic production and labelling of organic products (No 834/2007), states that livestock manure should be preferably composted before applying to the field in organic production (Council Regulation No 834/2007, article 12). Such statements are not available for non-organic production. MAP DNA has been found in composting material (cattle manure, sawdust/straw) (Grewal *et al*. 2006). Grewal *et al*. (2006) studied the persistence of MAP during composting at 55°C. They were not able to culture MAP after 3 days of composting but the insertion element IS*900* was found by PCR through day 56 from the composting material (Grewal *et al*. 2006).

10.3 Other ways of exposure

In addition to food and drinking water, other possible routes of human exposure to MAP are, for example, direct contact with the infected animals, exposure to contaminated environment through soil or water, person-to-person horizontal transmission and occupational exposure by farmers and veterinarians (Beumer *et al*. 2010, NACMCF 2010).

10.4 Methods to reduce the human exposure to *Mycobacterium avium* subsp. *paratuberculosis*

It is challenging to prevent the exposure of humans to MAP since the bacterium is shed to milk and feces of infected animals and subclinical infections are common (Whitlock & Buergelt 1996). The farm is therefore in critical role in reducing the human exposure to the bacterium by controlling the shedding of the bacterium (Eltholth *et al*. 2009).

Other ways to reduce the human exposure to MAP is by reducing the number of the bacteria in foods. The ways include for example pasteurization of milk, cooking of meat and water treatment techniques such as chlorination and ultraviolet irradiation (NACMCF 2010).

Whittington *et al*. (2010) noticed that temperatures used in cooking red meat are able to reduce the number of MAP in lamb significantly. Saucier & Plamondon (2011) boiled ground beef patties inoculated with MAP strains and came to a conclusion that normal cooking temperatures (70°C) are enough to control low concentrations of MAP in ground beef.

Chlorine is widely used in water treatment plants to produce safe drinking water since it kills most of the pathogenic bacteria (WHO 2011). The World Health Organization (WHO) recommends a guideline value for chlorine in water treatment to be 5.0 mg/l (5000 µg/ml) and the concentration of chlorine at the point of delivery to the customer should be at least 0.2 mg/l (200 µg/l) (WHO 2011). Throughout the world chlorine is present in most disinfected drinking water at concentrations of 0.2–1.0 mg/l (WHO 2011). In Helsinki region in Finland, the concentration of chlorine varies between 0.4–0.5 mg/l (HSY 2014a). In Minneapolis the concentration of chlorine in drinking water is about 3.0 mg/l (City of Minneapolis 2014b). Whan *et al*. (2001) found that chlorine used with levels of 0.5, 1.0 and 2.0 µg/ml (0.5, 1.0, 2.0 mg/l) did not kill all human and bovine strains of MAP when the cell concentration of MAP was 10^6 colony forming units (cfu)/ml. When they added bovine serum albumin to mimic organic matter often present in drinking water the effect of chlorine was reduced but the effectiveness was not significant (Whan *et al*. 2001).

Altic *et al*. (2007) studied the effect of UV radiation on the survival of MAP in semiskim and whole milk and noticed 0.5–1.0 $\log_{10}$ reductions in the number of MAP at 1000 mJ/ml. Donaghy *et al*. (2009) noticed 0.1–0.6 $\log_{10}$ reductions in the number of MAP in whole milk at 1000 mJ/ml.

The papers studying the heat resistance of MAP give widely differing results and since the studies have used very different conditions, the results are difficult to compare (Lund *et al*. 2002). Five of the nine studies reviewed by Lund *et al*. (2002) reported more than a 4 decimal reduction of MAP when the sample was heated at 72°C for 15 s (high temperature short time (HTST) pasteurization method). MAP has been found to be very persistent in the environment. MAP remained culturable in lake water microcosm for 632 days and the bacterium persisted detectable by real time PCR in the microcosm for 841 days (Pickup *et al*. 2005). Jørgensen (1977) found that MAP can survive viable in cattle and swine slurry in 5°C for 252 days and in cattle slurry for 98 days in 15°C. Whittington *et al*. (2004) cultured MAP from soil contaminated with sheep feces containing MAP after 55 weeks.

11 THE OCCURRENCE OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* IN FOOD AND WATER

The occurrence of MAP in food and water has been studied quite a lot. A compilation of the studies is represented in tables 5−9.

In asymptomatic animals, the infection status of the animals was not always known, as shown in Table 5. Therefore the results may be a bit difficult to compare and interpret. However, it can be noted that there are more positive results by PCR than by culture. That can be due to that PCR is a more sensitive detection method than culture and that culture of MAP requires decontamination of the samples. This might also reduce the number of MAP in the sample. Also PCR detects both dead and viable cells so it is natural that the percentage of MAP is higher by PCR than culture.

There are more positive results in the individual animals' results than in bulk tank milk results but that is logic since the bulk tank samples are taken from large tanks where the milk of several cows is mixed. Therefore the number of MAP might be too low for detection by the methods used. Raw bulk tank milk and retail pasteurized milk results can be assumed to be comparable with one another since the samples are both taken from large quantities of milk. The results vary a lot between studies. Some were not able to culture MAP at all or the percentages are small and others such as Javarao *et al.* (2004) were able to culture MAP in almost 1/3 of the samples from raw bulk tank milk. None of the papers used Finnish milk as a sample but MAP was found in several studies in the U.S. In general the occurrence of MAP in retail pasteurized milk is fairly low but still MAP has been able to culture in pasteurized milk in several studies.

Table 5 The occurrence of MAP in milk

Species	Country	Disease status	Sample type	Detection method	Positive samples No (%)	Detection limit	Reference
cow	UK	asymptomatic	raw bulk tank milk	IMS-PCR[a] (IS*900*)	19/244 (8)	1 cfu/50 ml	Grant *et al*. 2002
cow	UK	asymptomatic	raw bulk tank milk	culture (BACTEC[b], HEYM[c])	4/244 (2)	10 cfu/50 ml	Grant *et al*. 2002
cow	Ireland	asymptomatic	raw bulk tank milk	culture (BACTEC & HEYM)	0/310 (0)	100 cfu/50 ml	O'Doherty *et al*. 2002
cow	Spain	asymptomatic	raw bulk tank milk	PCR (IS*900*)	23/270 (9)	ND[d]	Sevilla *et al*. 2002
cow	Switzerland	asymptomatic	raw bulk tank milk	PCR (IS*900*)	112/501 (22)	ND	Stephan *et al*. 2002
cow	USA	asymptomatic	raw bulk tank milk	culture (HEYM)	6/29 (21)	ND	Jayarao *et al.* 2004
cow	USA	asymptomatic	raw bulk tank milk	PCR (IS*900*)	8/29 (28)	ND	Jayarao *et al.* 2004
cow	USA	asymptomatic	raw milk	culture (HEYM)	43/1493 (3)	ND	Jayarao *et al.* 2004
cow	USA	asymptomatic	raw milk	PCR (IS*900*)	201/1493 (13)	ND	Jayarao *et al.* 2004
cow	the Czech Republic	asymptomatic	raw milk	culture (HEYM)	15/483 (3)	ND	Ayele *et al*. 2005
cow	the Czech Republic	fecal culture negative	raw milk	culture (HEYM)	1/268 (0.4)	ND	Ayele *et al.* 2005
cow	Switzerland	asymptomatic	raw bulk tank milk	PCR (f57)	3/100 (3)	100 cfu/ml	Bosshard *et al*. 2006
cow	Iran	asymptomatic	raw bulk tank milk	nPCR[e] (IS*900*)	12/110 (11)	ND	Haghkhah *et al.* 2008
cow	Cyprus	asymptomatic	raw bulk tank milk[f]	culture (HEYM)	0/22 (0)	ND	Slana *et al*. 2009
cow	Cyprus	asymptomatic	raw bulk tank milk[f]	qPCR[g] (IS*900*)	49/220 (22)	5−6 cfu/ml	Slana *et al*. 2009

Table 5 Continued

Species	Country	Disease status	Sample type	Detection method	Positive samples No (%)	Detection limit	Reference
cow	Cyprus	asymptomatic	raw bulk tank milk[f]	qPCR (f57)	14/220 (6)	83 cfu/ml	Slana *et al*. 2009
cow	India	NK[h]	raw retail milk	culture (HEYM)	7/16 (44)	ND	Shankar *et al*. 2010
cow	India	NK[h]	raw retail milk	PCR (IS*900*)	1/16 (6)	ND	Shankar *et al*. 2010
cow	USA	fecal culture positive	raw milk	culture (HEYM)	9/77 (12)	ND	Sweeney *et al*. 1992
cow	Denmark	clinically infected	raw milk	culture (LJM[i])	5/11 (45)	100 cfu/ml	Giese & Ahrens 2000
cow	Denmark	clinically infected	raw milk	PCR (IS*900*)	2/11 (18)	1000 cfu/ml	Giese & Ahrens 2000
cow	USA	fecal culture positive	raw milk	PCR (IS*900*)	69/211 (33)	10−100 cfu/ml	Pillai & Jayarao 2002
cow	USA	fecal culture positive	raw milk	culture (HEYM)	9/211 (4)	10−100 cfu/ml	Pillai & Jayarao 2002
cow	USA	seropositive herd	raw bulk tank milk[j]	culture (HEYM)	0/52 (0)	< 10 cfu/ml	Stabel *et al*. 2002
cow	USA	seropositive herd	raw bulk tank milk[j]	PCR (IS*900*)	35/52 (67)	ND	Stabel *et al*. 2002
cow	USA	infected herd	raw bulk tank milk	PCR (IS*900*)	11/21 (52)	ND	Stabel *et al*. 2002
cow	USA	fecal culture positive	raw bulk tank milk	PCR (IS*900*)	10/20 (50)	10−100 cfu/ml	Pillai & Jayarao 2002
cow	USA	fecal culture positive	raw bulk tank milk	culture (HEYM)	1/20 (5)	10−100 cfu/ml	Pillai & Jayarao 2002
cow	USA, 10 states	infected herd	raw bulk tank milk	PCR (IS*900*)	24/31 (77)	ND	Stabel *et al*. 2002
cow	the Czech Republic	fecal culture positive	raw milk	culture (HEYM)	14/76 (18)	ND	Ayele *et al.* 2005

Table 5 Continued

Species	Country	Disease status	Sample type	Detection method	Positive samples No (%)	Detection limit	Reference
cow	Denmark	fecal culture positives & negatives	raw bulk tank milk	RT-PCR (IS*900*)	19/143 (13)	1−10 cfu/ml	Herthnek *et al*. 2008
cow	Mexico	seropositive	raw bulk tank milk	PCR (IS*900*)	14/14 (100)	ND	Favila-Humara *et al*. 2010
cow	Mexico	seropositive	raw bulk tank milk	culture (HEYM)	10/14 (71)	ND	Favila-Humara *et al*. 2010
cow	Mexico	seropositive	raw milk	PCR (IS*900*)	10/10 (100)	ND	Favila-Humara *et al*. 2010
cow	England & Wales	NK	retail pasteurized milk	culture (Dubos broth)	15/54 (28)	ND	Millar *et al*. 1996
cow	England & Wales	NK	retail pasteurized milk	PCR (IS*900*)	22/312 (7)	200−300 cfu/ml	Millar *et al*. 1996
cow	Canada	NK	retail pasteurized milk	culture (BACTEC)	0/244 (0)	ND	Gao *et al*. 2002
cow	Canada	NK	retail pasteurized milk	PCR (IS*900*)	110/710 (16)	100 cfu/ml	Gao *et al*. 2002
cow	UK	NK	commercially pasteurized milk	IMS-PCR (IS*900*)	67/567 (12)	1 cfu/50 ml	Grant *et al*. 2002
cow	UK	NK	commercially pasteurized milk	culture (HEYM, BACTEC)	10/567 (2)	10 cfu/50 ml	Grant *et al*. 2002
cow	Ireland	NK	retail pasteurized milk	culture (BACTEC & HEYM)	0/77 (0)	100 cfu/50 ml	O'Doherty *et al*. 2002
cow	the Czech Republic	NK	commercially pasteurized milk	culture (HEYM)	4/244 (2)	ND	Ayele *et al*. 2005
cow	the Czech Republic	NK	locally pasteurized milk	culture (HEYM)	2/200 (1)	ND	Ayele *et al*. 2005

Table 5 Continued

Species	Country	Disease status	Sample type	Detection method	Positive samples No (%)	Detection limit	Reference
cow	USA (CA, MN, WI[k])	NK	retail pasteurized milk	culture (HEYA, ESP[l]) & confirmatory PCR (IS*900* and *hspX*)	20/702 (3)	ND	Ellingson *et al*. 2005
cow	USA, Minnesota	NK	retail pasteurized milk	culture (HEYA, ESP) & confirmatory PCR (IS*900* and *hspX*)	9/234 (4)	ND	Ellingson *et al*. 2005
cow	USA (CA, MN, WI)	NK	retail pasteurized milk	PCR (IS*900* and *hspX*)	452/702 (64)	1 cfu/reaction (IS*900*), 20 cfu/reaction (*hspX*)	Ellingson *et al*. 2005
cow	India	NK	retail pasteurized milk	PCR (IS*900*)	7/18 (39)	ND	Shankar *et al*. 2010
cow	India	NK	retail pasteurized milk	culture (HEYM)	13/18 (72)	ND	Shankar *et al*. 2010
goat	Ireland	NK	retail pasteurized milk	culture (BACTEC & HEYM)	0/9 (0)	100 cfu/50 ml	O'Doherty *et al*. 2002
goat	Norway	vaccinated animals, JD reported	raw milk	IMS-PCR (dot blot technique)	4/120 (3)	0.1 cfu/ml	Djønne *et al*. 2003[m]
goat	Norway	vaccinated animals, JD never reported	raw milk	IMS-PCR (dot blot technique)	11/100 (11)	0.1 cfu/ml	Djønne *et al*. 2003[m]
goat	Norway	not vaccinated animals, JD never reported	raw milk	IMS-PCR (dot blot technique)	9/120 (8)	0.1 cfu/ml	Djønne *et al*. 2003[m]
goat	Norway	not vaccinated animals, JD never reported	raw milk	culture	0/340 (0)	10 cfu/ml	Djønne *et al*. 2003[m]
goat	Switzerland	NR[n]	raw bulk tank milk	PCR (IS*900*)	79/344 (23)	ND	Muehlherr *et al*. 2003

Table 5 Continued

Species	Country	Disease status	Sample type	Detection method	Positive samples No (%)	Detection limit	Reference
goat	India	clinically infected	raw milk	culture (HEYM)	1/10 (10)	ND	Singh & Vihan 2004
goat	Italy	seropositive herd	raw milk	nPCR (IS*900*)	6/9 (67)	ND	Nebbia *et al.* 2006
goat	Greece	NR	raw bulk tank milk	qPCR (IS*900*)	0/13 (0)	<10 cfu/ml	Botsaris *et al*. 2010
goat	Greece	NR	raw bulk tank milk	culture (HEYM)	0/13 (0)	ND	Botsaris *et al*. 2010
goat	Mexico	seropositive	raw bulk tank milk	PCR (IS*900*)	3/3 (100)		Favila-Humara *et al*. 2010
goat	Mexico	seropositive	raw bulk tank milk	culture	3/3 (100)	ND	Favila-Humara *et al*. 2010
goat	Mexico	seropositive	raw milk	PCR (IS*900*)	8/8 (100)	ND	Favila-Humara *et al*. 2010
sheep	Switzerland	NR	raw bulk tank milk	PCR (IS*900*)	15/63 (24)	ND	Muehlherr *et al*. 2003
sheep	Australia	clinically infected	raw milk	culture (BACTEC)	2/76 (3)	ND	Lambeth *et al*. 2004
sheep	Italy	seropositive herd	raw milk	nPCR (IS*900*)	7/20 (35)	ND	Nebbia *et al.* 2006
sheep	Greece	NR	raw bulk tank milk	qPCR (IS*900*)	1/5 (20)	<10 cfu/ml	Botsaris *et al*. 2010
sheep	Greece	NR	raw bulk tank milk	culture (HEYM)	0/5 (0)	ND	Botsaris *et al*. 2010
sheep & goat	Greece	NR	raw bulk tank milk	qPCR (IS*900*)	1/19 (5)	<10 cfu/ml	Botsaris *et al*. 2010
sheep & goat	Greece	NR	raw bulk tank milk	culture(HEYM	0/19 (0)	ND	Botsaris *et al*. 2010

[a]IMS Immunomagnetic separation combined with PCR
[b]BACTEC Radiometric culture
[c]HEYM Herrold Egg Yolk Medium
[d]ND No detection limit was identified
[e]nPCR Nested PCR
[f]All the dairy herds within the country
[g]qPCR Quantitative PCR
[h]NK Not known
[i]LJM Löwenstein-Jensen medium
[j]Either fecal culture positives or negatives
[k]California, Wisconsin & Minnesota
[l]ESP culture method
[m]No decontamination methods used
[n]NR Not reported

In cheese the culture of MAP has not been really successful, as can be seen in Table 6. Either the amount of samples has been small or MAP has been able to culture only on one or two samples per one study. The reasons for this might be due to the long ripening process of cheese. It could be possible that MAP is destroyed during the ripening and thus only MAP DNA is found. An interesting fact is that MAP has been found in infant milk powder. If MAP is found to be an etiological agent of CD, special attention should be addressed on infant and children's food because it might be that small children are more susceptible to mycobacterial infections than adults.

In addition to milk and cheese, MAP has been cultured from other dairy products as well. Shankar *et al*. (2010) cultured MAP from pasteurized ice cream and liquid dairy products from 5/9 (56%) of the samples and found IS*900* sequence specific to MAP in 2/9 (22%) of the samples. One possible route of exposure to MAP could be by infant milk powder since milk is used in a variety of infant foods. Hruska *et al*. (2005) tested 51 different dried milk infant products from ten producers. The producers came from seven European Union countries (Hruska *et al*. 2005). IS*900* sequence was found in 25 samples (49%) and fragment f57 was found in 18 samples (35%) (Hruska *et al*. 2005). Botsaris *et al.* (2012) were able to culture MAP from 9% of infant formula samples bought from Cyprus and they found IS*900* sequence in 22% of the samples. Rowe *et al*. (2007) on the other hand were not able to culture MAP from milk powder but they found IS*900* sequence in 18/190 (9%) of the samples.

Table 6 The occurrence of MAP in cheese

Type of cheese	Species	Country	Pasteur-ized	Detection method	Positive samples No (%)	Detection limit cfu/ml	Reference
feta	NR[a]	Greece	yes	PCR (IS*900*)	21/42 (50)	ND[b]	Gazouli *et al*. 2003
feta	NR	Greece	yes	culture (HEYM[c], BACTEC[d])	1/42 (2)	ND	Gazouli *et al*. 2003
ND	NR	the Czech Republic	yes	PCR (IS*900*)	5/42 (12)	ND	Ikonomopoulos *et al*. 2005
ND	NR	the Czech Republic	yes	culture (HEYM)	1/43 (2)	ND	Ikonomopoulos *et al*. 2005
feta	sheep & goat	Greece	yes	PCR (IS*900*)	21/42 (50)	ND	Ikonomopoulos *et al*. 2005
feta	sheep & goat	Greece	yes	culture (HEYM)	2/42 (5)	ND	Ikonomopoulos *et al*. 2005
cheese curds	cow	USA (MI, WI)	yes	PCR (IS*900*)	23/98 (23)	ND	Clark *et al*. 2006
cheese curds	cow	USA (MI, WI)	yes	PCR (*hspX*)	9/98 (9)	ND	Clark *et al*. 2006
cheese curds	cow	USA (MI, WI)	yes	culture (HEYA)	0/98 (0)	ND	Clark *et al*. 2006
soft, semihard, hard	cow	Switzerland	no	RT-PCR[e] (f57)	6/143 (4)	ND	Stephan *et al.* 2007
soft, semihard, hard	cow	Switzerland	no	culture (7H10-PANTA[f], BACTEC)	0/143 (0)	50	Stephan *et al.* 2007
retail traditional cheese	cow, sheep & goat	Greece	yes	qPCR[g] (IS*900*)	7/28 (25)	ND	Botsaris *et al*. 2010
retail traditional cheese	cow, sheep & goat	Greece	yes	culture (HEYM)	0/28 (0)	ND	Botsaris *et al*. 2010
semihard, hard	cow	Scotland	no	culture (HEYM, Middlebrook)	2/20 (10)	ND	Williams & Withers 2010
semihard, hard	sheep	Scotland	no	culture (HEYM, Middlebrook)	3/4 (75)	ND	Williams & Withers 2010
semihard, hard	goat	Scotland	no	culture (HEYM, Middlebrook)	0/1 (0)	ND	Williams & Withers 2010
semihard, hard	cow	Scotland	yes	culture (HEYM, Middlebrook)	2/3 (67)	ND	Williams & Withers 2010

[a]NR Not reported
[b]ND Not determined
[c]HEYM Herrold Egg Yolk Medium
[d]BACTEC Radiometric culture
[e]RT-PRC Real-Time PCR
[f]Middlebrook 7H10 agar with antibiotic supplement
[g]qPCR Quantitative PCR

Studies conducted on the occurrence of MAP in meat have mostly concentrated on beef but also some studies on mutton have been done, shown in Table 7. A lot of the studies have been conducted on infected animals. The prevalence of JD is high in the countries where the studies have been made; therefore it is logic that also the animals used in the studies have an infection. Thus it is more relevant to estimate the exposure of humans to MAP through meat based on these studies. Wells *et al.* (2009) were able to culture MAP from 172/338 (51%) of carcass swab samples after the removal of the hide but before any interventions but only from 3/302 (1%) samples postintervention. This suggests that the postintervention methods used are effective on the destruction of MAP. Thus it could be that at a slaughter plant, well executed processing of the carcass might prevent humans from being extensively exposed to MAP through meat. Many studies have included lymph nodes in the samples because they are often used in ground beef.

In addition to beef, MAP has also been found in goat meat. Manning *et al.* (2003) cultured MAP from clinically infected goats' kidneys and hindlimb muscles from 4/10 (40%) and from 2/10 (20%) of the samples, respectively.

Klanicova *et al.* (2011) studied also the occurrence of MAP in cooked pork and chicken and they found MAP DNA from 14% of pork samples and from 50% of chicken samples. They were not able to culture MAP from any of the samples. Swine and poultry don't have JD so the results suggest contamination of the products in the processing phase. Thus it is extremely important that the processing phase of any product is done according to good hygiene standards.

Table 7 The occurrence of MAP in beef

Country	Type of cattle	Disease status	Sampling site	Detection method	Positive samples No (%)	Detection limit	Reference
USA	beef	sound[a]	liver	culture (Cornell double incubation)	1/350 (0.3)	ND[b]	Rossiter & Henning 2001
USA	beef	sound[a]	superficial & popliteal lymph nodes	culture (Cornell double incubation)	1/350 (0.3)	ND	Rossiter & Henning 2001
USA	dairy	sound[c]	liver	culture (Cornell double incubation)	15/189 (8)	ND	Rossiter & Henning 2001
USA	dairy	sound[c]	superficial & popliteal lymph nodes	culture (Cornell double incubation)	6/189 (3)	ND	Rossiter & Henning 2001
USA, California	NK	NK	retail ground beef	RT-PCR[d] (IS*900*)	0/200 (0)	10^1 cfu/g	Jaravata *et al*. 2007
North America	fed	NR[e]	carcass swab, anal region, after skinning	nPCR[f] (IS*900*, f57, IAC[g])	43/98 (44)	ND	Meadus *et al*. 2008
North America	beef	NR	carcass swab, anal region, after dressing	nPCR (IS*900*, f57, IAC)	24/100 (24)	ND	Meadus *et al*. 2008
USA	fed	NR	carcass swab	culture (HEYA[h])	0/455 (0)	ND	Wells *et al*. 2009
USA	cull	NR	carcass swab, preevisceration	culture (HEYA)	172/338 (51)	ND	Wells *et al*. 2009
USA	cull	NR	carcass swab, postintervention	culture (HEYA)	3/302 (1)	ND	Wells *et al*. 2009
USA	fed	NR	ileocecal lymph nodes	PCR (IS*900*)	1/232 (0.4)	ND	Wells *et al*. 2009
USA	cull	NR	ileocecal lymph nodes	PCR (IS*900*)	113/330 (34)	ND	Wells *et al*. 2009
USA	fed	NR	hides	PCR (IS*900*)	3/243 (1)	ND	Wells *et al*. 2009
USA	cull	NR	hides	PCR (IS*900*)	273/343 (80)	ND	Wells *et al*. 2009
USA, California	dairy	disseminated infection[i]	muscle (longissimus colli, extensor carpi radialis),	culture (HEYM[j])	0/21 (0)	ND	Antognoli *et al*. 2008

Table 7 Continued

Country	Type of cattle	Disease status	Sampling site	Detection method	Positive samples No (%)	Detection limit	Reference
USA, California	dairy	disseminated infection	liver	culture (HEYM)	10/21 (48)	ND	Antognoli *et al*. 2008
USA, California	dairy	disseminated infection	kidney	culture (HEYM)	6/21 (29)	ND	Antognoli *et al*. 2008
Spain	dairy and beef[k]	infected herds	muscle, diaphragm	culture (HEYM, LJM[l])	6/47 (13)	ND	Alonso-Hearn *et al.* 2009[m]
Spain	dairy and beef[k]	infected herds	mesenteric lymph nodes	culture (HEYM, LJM)	23/47 (49)	ND	Alonso-Hearn *et al.* 2009[m]
Canada	dairy	clinically infected	liver, lymph nodes,	culture (HEYA, MGIT[n])	7/15 (47)	4 cfu	Mutharia *et al.*2009
Canada	dairy	clinically infected	kidneys, lymph nodes	culture (HEYA, MGIT)	5/15 (33)	1 cfu	Mutharia *et al.*2009
Australia	NR	clinically infected	muscle (rump, forequarter)	culture (BACTEC[o])	1/9 (11)	1.77 ± 0.4 $\log_{10}$ organisms per gram of meat	Reddacliff *et al*. 2010[p]
Australia	NR	clinically infected	lymph nodes	culture (BACTEC)	5/9 (55)	1.77 ± 0.4 $\log_{10}$organisms per gram of meat	Reddacliff *et al*. 2010[p]

[a]Body score <4 (1−9)
[b]ND No detection limit was identified
[c]Body score <2.5 (1−5)
[d]RT-PCR Real-Time PCR
[e]NR Not reported
[f]nPCR Nested PCR
[g]IAC – internal amplification control
[h]HEYA Herrold Egg Yolk Agar
[i]Disseminated infection determined as MAP isolated in tissues other than intestine and associated lymph nodes
[j]HEYM Herrold Egg Yolk Medium
[k]Forty-two dairy cows, five beef cows
[l]LJM Löwenstein-Jensen medium
[m]26% of the animals had clinical signs of JD
[n]MGIT Mycobacteria Growth Indicator Tube
[o]BACTEC Radiometric Culture
[p]Acid-pepsin digestion technique used as a decontamination method

Table 8 The occurrence of MAP in mutton

Country	Disease status	Sampling site	Detection method	Positive samples No (%)	Detection limit	Reference
Australia	uninfected animals	supramammary lymph nodes, uterus;	culture (BACTEC[a])	4/136 (3)	ND[b]	Lambeth *et al.* 2004
Australia	subclinical infection	muscle (rump, forequarter),	culture (BACTEC)	1/22 (5)	1.77 ± 0.4 $\log_{10}$organisms per gram of meat	Reddacliff *et al*. 2010[c]
Australia	subclinical infection	peripheral lymph nodes	culture (BACTEC)	7/22 (32)	1.77 ± 0.4 $\log_{10}$organisms per gram of meat	Reddacliff *et al*. 2010[c]
New Zealand	subclinical infection	muscle, biceps femoris	culture (BACTEC)	4/30 (13)	ND	Smith *et al*. 2011
Australia	heavily infected herd	fetus[d,e]	culture (BACTEC)	2/119 (2)	ND	Lambeth *et al.* 2004
Australia	clinical cases	mammary gland,	culture (BACTEC)	2/136 (1)	ND	Lambeth *et al.* 2004
Australia	clinically infected	muscle (rump, forequarter),	culture (BACTEC)	20/34 (59)	1.77 ± 0.4 $\log_{10}$organisms per gram of meat	Reddacliff *et al*. 2010[c]
Australia	clinically infected	peripheral lymph nodes,	culture (BACTEC)	29/34 (85)	1.77 ± 0.4 $\log_{10}$organisms per gram of meat	Reddacliff *et al*. 2010[c]
Australia	infected herd	mesenteric lymph nodes	culture (BACTEC)	44/46 (96)	ND	Dennis *et al.* 2011
New Zealand	clinically infected	muscle, biceps femoris,	culture (BACTEC)	5/21 (24)	ND	Smith *et al*. 2011

[a]BACTEC Radiometric Culture
[b]ND No detection limit was identified
[c]Acid-pepsin digestion technique used as a decontamination method
[d]Both positive samples were from ewes with clinical signs
[e]Samples from liver, spleen, umbilicus

The occurrence of MAP in plants has only been studied in one study. In the study, Pribylova *et al*. (2011) used a field with deliberately exposed to MAP infected mouflon feces. The feces were deposited on the field by the animals themselves and the plants were examined 15 weeks after exposure to the feces (Pribylova *et al*. 2011). MAP was not cultured on the plant parts but IS*900* sequence was detected in 13/19 (68%) of green upper parts of the plants and in 15/19 (79%) of the roots of the plants (Pribylova *et al*. 2011).

MAC is often found in drinking water systems but Table 8 only represents MAP found in drinking water and drinking water systems. From Table 8, it is easy to notice that MAP DNA is frequently found in raw surface water but also from drinking water systems and tap water. It seems to survive well of the normal drinking water treatments. Mishina *et al.* (1996) cultured from municipal water supply from a major city in the United States for the first time in 1996. The study didn't specify the name of the city in question. In addition, only 2 other studies had positive results on culture of MAP. That might be due to the difficultness of culturing MAP or that viable MAP are not present in drinking water at such numbers as MAP DNA. Also the sensitivity of PCR is greater than that of culture method.

Table 9 The occurrence of MAP in water and drinking water systems

Country	Sample	Detection method	Positive samples No (%)	Detection limit	Reference
USA	municipal water supply	culture	NA[a,b] (NA)	ND[c]	Mishina *et al*. 1996
UK	river water	culture	12/96 (13)	ND	Pickup *et al.* 2005
UK	river water	qPCR[d](IS*900*)	31/96 (32)	ND	Pickup *et al.* 2005
Northern Ireland	water treatment plant	culture (BACTEC[e] or HEYM[f])	8/192 (4)	ND	Whan *et al.* 2005
Northern Ireland	water treatment plant	IMS-PCR[g] (IS*900*)	9/192 (5)	ND	Whan *et al.* 2005
UK	river water	PCR (IS*900*)	48/70 (69)	ND	Pickup *et al.* 2006
UK	household cold water tank sediment	PCR (IS900)	1/54 (2)	ND	Pickup *et al.* 2006
UK	finished drinking water 100 liters	PCR (IS*900*)	0/1 (0)	ND	Pickup *et al.* 2006
USA, 25 states	drinking water	qPCR (IS*900*)	5/238 (0.02)	ND	Beumer *et al*. 2010
USA, Midwest	drinking water	qPCR (IS*900* and target 251)	29/33 (88)	ND	Beumer *et al*. 2010
USA, Midwest	faucet biofilm	qPCR (IS*900* and target 251)	25/33 (76)	ND	Beumer *et al*. 2010
Northern Ireland	raw surface water	PCR (IS*900*)	27/48 (56)	10 cfu/ml	Aboagye *et al.* 2011
Northern Ireland	raw surface water	PCR (f57)[h]	7/27 (26)	10^3 cfu/ml	Aboagye *et al.* 2011
Northern Ireland	treated water	PCR (IS*900*)	20/43 (47)	10 cfu/ml	Aboagye *et al.* 2011
Northern Ireland	treated water	PCR (f57)[h]	1/20 (5)	10^3 cfu/ml	Aboagye *et al.* 2011
Northern Ireland	treated water	culture (BACTEC)	1/NA (NA)	30 cfu/plate	Aboagye *et al.* 2011
Italy	drinking water	snPCR[i] & nPCR[j] (f57 & IS*900*)	3/90 (3)	ND	Pistone *et al.* 2012
India	river water	microscopic examination	6/20 (30)	ND	Singh *et al.* 2012
India	river water	PCR (IS*900*)	2/20 (10)	ND	Singh *et al.* 2012
the Czech Republic	reservoir sediment	Duplex qPCR (IS*900*)	36/52 (69)	10^2 copies/g	Klanicova *et al.* 2013
the Czech Republic	water treatment sludge	Duplex qPCR (IS*900*)	7/34 (21)	10^2 copies/g	Klanicova *et al.* 2013
the Czech Republic	household sediment	Duplex qPCR (IS*900*)	2/38 (5)	10^2 copies/g	Klanicova *et al.* 2013
UK	river Douglas	qPCR (IS*900*)	ND (42)	0.1−1 cell equivalents	Rhodes *et al.* 2013
UK	river Wyre	qPCR (IS*900*)	ND (36)	0.1−1 cell equivalents	Rhodes *et al.* 2013

[a]NA Not available
[b]MAP was cultured but the study didn't specify exact numbers
[c]ND No detection limit was identified
[d]qPCR Quantitative PCR
[e]BACTEC Radiometric culture
[f]HEYM Herrold Egg Yolk Medium
[g]IMS-PCR Immunomagnetic separation combined with PCR
[h]f57 was tested only on IS*900* positive samples
[i]snPCR Seminested PCR
[j]nPCR Nested PCR

12 ORIGIN, CONSUMPTION AND IMPORT OF FOOD IN FINLAND

12.1 Origin of food

12.1.1 Import of cattle

In 2012, 46 live cattle were imported to Finland. Of those 33 cattle were from Sweden, three from Denmark and ten from Scotland (ETT 2014a).

12.1.2 Drinking water

In Finland, 42% of drinking water comes from surface water and 58% from groundwater and artificial groundwater (Hänninen 2007). Drinking water in the Helsinki metropolitan area (cities of Helsinki, Vantaa, Espoo, Kauniainen) to 1.2 million people, is taken from Lake Päijänne (HSY 2014b). Also small amounts of ground water and water from Lake Pitkäjärvi are used in the Helsinki metropolitan area (HSY 2014b).

12.2 Consumption and import of food

12.2.1 Consumption of food

The figures for the annual consumption of foods and the share and amount of imported food of all consumed in Finland in 2012 per capita are represented in Table 10. The figures are calculated based on the production statistics presented in the Balance sheet for food commodities 2012, from the Information Centre of the Ministry of Agriculture and Forestry, Tike (Tike 2014c). The consumption of lamb and mutton, goat meat and goat cheese are not represented due to the low consumption (< 0.5 kg per capita annually). Also the consumption of vegetables is not represented because the lack of evidence of the occurrence of MAP in this food group.

Table 10 Annual consumption per capita of selected food groups and share of imported food of all consumed food in Finland in 2012

Product	Annual consumption per capita, kg	Imported food of all consumed, kg (%)	Reference
Beef and veal (inc. home slaughter)	18.7	4.0 (21.6)	Tike 2014c,d
Liquid dairy (incl. cream, curdled milk, sour milk)	159.2	12.3 (7.7)	Tike 2014d, Uljas 2014
Raw milk	1.7	NA[a]	Tike 2014c
Cheese	21.9	11.3 (51.6)	Tike 2014c,d
Yogurt	23.3	6.5 (27.9)	Tike 2014c,d

[a]NA Not available

The consumption of imported beef and veal is rising in Finland because Finnish cattle production is diminishing every year (Table 10, Tike 2014a). The annual production has decreased about 15 million kg in ten years, from almost 96 million kg in 2003 to 81 million kg in 2013 (Tike 2014a). The consumption of beef on the other hand has stayed around 18−19 kg per capita annually (Tike 2014d). For example, in 2010 the amount of imported beef and veal of all consumed was 2.3 kg per capita so it has almost doubled in just two years (Tike 2014a,b). The share of imported cheese of all cheese consumed is also rising, from 8.9 kg per capita in 2010 to 11.3 kg per capita in 2012 (Table 10, Tike 2014c). This is explained both by the increase in consumption of cheese and by increase in import of cheese to Finland (Tike 2014c,d).

12.2.2 Import of food

In 2102, beef was mostly imported from Poland, the Netherlands, Germany and Denmark (Uljas 2014). Over 51 million kilograms of milk and cream were imported to Finland from Sweden in 2012 representing 77 % of all imported milk that year (Uljas 2014). The rest was mostly imported from Germany and Estonia (Uljas 2014). Cheese

was imported from Germany, Denmark and Sweden (Uljas 2014). The biggest yogurt importing countries in 2012 were Germany, Poland and Estonia (Uljas 2014).

13 ORIGIN, CONSUMPTION AND IMPORT OF FOOD IN THE UNITED STATES

13.1 Origin of drinking water

In the United States, 80% of all water used comes from surface water and 20% from groundwater (Barber 2005). In Minnesota, over 70% of the drinking water consumed is groundwater (Water System Council 2014). Drinking water in Minneapolis comes from the Mississippi River (City of Minneapolis 2014a).

13.2 Consumption and import of food

13.2.1 Consumption of food

There are no statistics about the consumption of raw milk in the United States. In a survey made by the Centers for Disease Control over 17,000 people in 10 states were asked about their food consumption (CDC 2006–2007). Of these people, 3% answered that they had drunk unpasteurized (raw) milk during the past seven days. The percentage was 2% in Minnesota (CDC 2006–2007). In Minnesota, consumers are occasionally allowed to buy unpasteurized, raw cow's, goat's or sheep's milk for personal use from the place or farm where the milk is produced (Minnesota Statutes 2013).

The figures for the annual consumption of foods in the United States per capita in 2009 are represented in Table 11.

Table 11 Annual consumption of foods in the United States per capita in 2011

Beef, kg	Liquid dairy products, kg	Cheese, kg	Yogurt, kg	Reference
26.0	84.5	15.0	6.2	USDA − ERS 2014a,b,c

13.2.2 Import of beef and veal

The biggest beef and veal importing countries in the US are Australia, Canada, New Zealand and Brazil (USDA – ERS 2014d). For example in Australia JD is present in dairy herds the southern part of the country including Tasmania but they have implemented an effective control program to prevent the spread of the disease between states and between dairy and cattle herds (Animal Health Australia 2014). The share of imported beef and veal of the total beef supply in the US was about 9% in 2012 (USDA – ERS 2014d). Of all imported beef and veal, about 30% came from Australia in 2012 (USDA – ERS 2014d).

14 DISCUSSION

There are several uncertainties about the etiology of CD. The possible role of MAP in the development of the disease has been suggested already over 100 years ago but the association between MAP and CD is difficult to prove reliably. MAP is widely present in the environment; therefore humans are exposed to the bacterium for example through food and drinking water and by occupational exposure. MAP is also found in CD patients' tissues more often than from healthy controls' tissues. At the moment it is not known whether MAP is just a commensal bacterium in the gut of humans or indeed a human pathogen. It could be possible that subgroups of patients with different genotypes are more susceptible to some etiological agents of CD than others and therefore not only one type of disease could exist. Also, if MAP was a pathogenic bacterium to humans, the infective dose of the bacterium would be unknown.

MAP is very durable in the environment and it can survive standard pasteurization methods. It is thus possible that MAP could be one of the etiological agents of CD but controversy exists. MAP is frequently found in food and drinking water and the exposure to MAP can be estimated but it is still unknown if MAP present in food is able to infect people. The detection of MAP from food is more common by PCR than by culture in dairy products. PCR detects MAP DNA, therefore both dead and viable MAP are found. It is unknown if also MAP DNA from dead cells is able to elicit inflammation in humans.

JD is a globally important disease of cattle and other ruminants and it causes massive economic impacts to the cattle industry throughout the world. The prevalence of JD in cattle is low in only few places in the world as in the Nordic countries. In general, the prevalence of JD is high in the United States. Studies conducted on national level have found the prevalence of JD to be much lower in beef cattle than in dairy cattle. Beef cattle are sent to slaughter before two years of age. The incubation period of JD is very long and thus it is rare that beef cattle present clinical signs of JD while being raised at the farm. Sensitivity of ELISA has been found to be only about 15% to detect subclinical cases of JD. That is why the true prevalence of JD in beef cattle is presumably much higher than the apparent prevalence. The prevalence of JD in dairy

cattle is higher on national level than in Minnesota but still about half of all Minnesotan dairy herds are positive for JD.

In Finland the situation is completely different since only few positive cases of JD in beef cattle have been found in Finland since the beginning of the 90's. No positive cases in dairy cattle have been found. Since the disease is so rare, it might be possible that veterinarians don't include the disease on their differential list of diseases when diagnosing cattle with emaciation and diarrhea. Also dairy cows are sent to slaughter at a fairly young age in Finland so seeing a clinical case of JD in dairy cows would be a rare occasion. Thus it might be possible that the true prevalence of JD is higher than previously thought in Finland but the importance of the disease is still quite small. Import of cattle to Finland is low and majority of live cattle imported are from Sweden where the prevalence of JD is equally low as in Finland. Also the import requirements are quite tight. Therefore the odds of that the disease would spread to Finland through imported animals seems to be minimal.

The occurrence of MAP in drinking water has not been studied in Finland. It is not likely that Finnish people are exposed to MAP through drinking water due to the low prevalence of JD in the country. Finnish people eat mostly food of Finnish origin but in some food groups the proportion of imported food is high, such as in cheese, yogurt and beef. The prevalence of JD in the biggest importing countries, such as in Germany and Denmark is very high. Therefore it is likely that Finns who eat a lot of imported food in these food groups are exposed to the bacterium in a much larger scale compared to Finns only eating food of Finnish origin or only a little of imported food. Even though Finland is quite self-sufficient in many food products such as liquid milk; new processed foods, such as functional food are becoming more and more popular among consumers. This might create new needs importing more food in these food groups. Also the import of food in certain food groups is rising. These facts might also increase the exposure of Finnish people to MAP. Nevertheless, based on this literature review it can be noted that the exposure of humans to MAP in Finland through food and drinking water is low.

Viable MAP have been found in drinking water in the United States. The source of drinking water in the country varies a lot but in Minnesota 30% of the drinking water comes from surface water. There is a bigger risk that surface water contains more MAP than groundwater since the surface water is not purified naturally when it goes through the layers of the ground like groundwater does. Runoffs from farms can get into drinking water. There are a lot of dairy and beef farms in Minnesota and runoffs from these farms can result in contamination of surface water resources. Because the prevalence of JD is so high in the state, it is possible that MAP is present in drinking water in Minnesota and that people are exposed to the bacterium. Drinking water from surface sources in the United States is chlorinated but the effectiveness of chlorination regarding killing MAP has been found to be ineffective.

The share of imported beef is less than 10% in the United States. Even though about one third of beef is imported from Australia where JD is not endemic in the whole country, the large majority of beef consumed in the United States is produced there and therefore it is likely that people eating beef in the United States are exposed to MAP. The exposure to MAP can be limited or prevented though if the slaughtering process is executed with high hygiene standards and meat is cooked and handled correctly at home. In Minnesota, the exposure of humans to MAP through food is at least moderate because the prevalence of JD is quite high and the consumption of foods that are proven to contain MAP such as liquid dairy products is quite high. Based on this thesis it can be stated that people in Minnesota are exposed to MAP through food and drinking water in a much larger scale than Finnish people since the prevalence of JD in Minnesota and in the United States is so high.

The prevalence and incidence of CD have been increasing globally in the past 70 years. The reasons for this are unknown but possible explanations are actual increase of incidence and the ever more developing diagnostic methods of medicine. The prevalence of CD is higher in Minnesota than in Finland in adult patients. Regardless of this, the prevalence of CD in Finland is also among the highest reported in the world. CD has been studied more in Minnesota than in Finland and this might partly may explain the differences. Finland and Minnesota are situated on different latitudes and they both share a fairly similar climate. They both have a population of about 5 million and they both have high proportion of white inhabitants and high level of education. In

the late 19th century and early 20th century a large number of Finns immigrated to Minnesota. The similar demographic and climatic characteristics of Minnesota and Finland might explain the high prevalence of CD in these geographical areas if they shared environmental factors that have an impact in the development of CD. But that kind of geographical comparison has not been done and it is not known if this hypothesis is true.

The prevalence of JD is very high in some European countries such as Germany and Denmark but the prevalence and incidence of CD is about the same in these countries and Finland. If MAP was an etiological agent of CD, it would be intriguing to discover the pathogenesis of CD in Finnish patients because of the low exposure of Finnish people to MAP compared to many other countries. What would be the reason of the high prevalence of CD in Finland if it wasn't MAP?

15 CONCLUSIONS

This thesis supports the hypothesis that people in areas of high prevalence of JD are exposed more to MAP than people in areas of low prevalence of JD. Based on this literature review it can be assumed that in Finland CD would be caused by some other environmental agent than MAP. To prove this statement, further research is required. The prevalence of JD in Finland has to be studied with larger scale studies than previously done. Also the occurrence of MAP in Finnish foods has to be studied.

This kind of literature review has not been done previously. Comparing subsets of CD patients with high exposure to MAP to controls with and without of human exposure to MAP through food and drinking water could help reveal the possible role of MAP in the complex etiology of CD. This thesis sets up further research needed to be done in estimating the true exposure of humans to MAP in geographic areas with different prevalence of JD.

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